Sterile Drug Products Produced by Aseptic Processing

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I. INTRODUCTION

II. BACKGROUND

There are basic differences between the production of sterile drug products by aseptic processing and by terminal sterilization.

Terminal sterilization usually involves filling and sealing product containers under conditions of a high quality environment; the product, container, and closure in most cases have low bioburden but are not sterile. The environment in which filling and sealing is performed is of high quality in order to minimize the microbial content of the in-process product, and to help ensure that the subsequent sterilization process is successful. The product in its final container is then subjected to a sterilization process such as heat or radiation.

In aseptic processing, the drug product, container, and closure are subjected to sterilization processes separately, as appropriate, and then brought together.[1] Because there is no further processing to sterilize the product after it is in its final container, it is critical that containers be filled and sealed in an environment of extremely high quality. Manufacturers should be aware that there are more variables associated with aseptic processing than terminal sterilization. Before aseptic assembly, different parts of the final product are generally subjected to different sterilization processes, such as dry heat for glass containers, moist heat sterilization for rubber closures, and sterile filtration for a liquid dosage form. Each of the processes of the aseptic manufacturing operation requires thorough validation and control. Each also introduces the possibility of error that might ultimately lead to the distribution of contaminated product. Any manual or mechanical manipulation of the sterilized drug, components, containers, and closures prior to or during aseptic assembly poses a risk of contamination and thus necessitates careful control. The terminally sterilized drug product, on the other hand, undergoes a single sterilization process in a sealed container, thus limiting the possibilities for error.[2]

Manufacturers should have a keen awareness of the public health implication of distributing a non-sterile drug purporting to be sterile. Poor CGMP conditions at a manufacturing facility can ultimately pose a life threatening health risk to a patient.

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[1] Due to their nature, certain products are aseptically processed from an earlier stage in the process, or in their entirety. Cell-based therapy products are an example. All components and excipients for these products are rendered sterile, and release of the final product is contingent on determination of sterility. See Appendix III.

[2] Nearly all drugs recalled due to Non-sterility or Lack of Sterility Assurance in the period spanning 1980-2000 were produced via aseptic processing.

III. SCOPE

This document discusses only selected issues and thus does not address all aspects of aseptic processing. Finished drug product CGMP issues are primarily addressed, with only limited guidance regarding upstream bulk processing steps. Updates relative to the 1987 document include guidance on: personnel qualification, clean room classifications under dynamic conditions, room design, quality control, environmental monitoring, and review of production records. The aseptic processing isolator is also discussed.

Although this document discusses CGMP issues relating to the sterilization of components, containers, and closures, terminal sterilization of the drug product is not addressed. It is a well-accepted principle that sterile drugs should be manufactured by aseptic processing only when terminal sterilization is not feasible. However, unacceptable degradation of the product can occur as a result of terminal sterilization, or the market presentation can afford some unique and substantial clinical advantage not possible if terminal sterilization were employed. In such cases, adjunct processing steps (e.g., heat exposure conditions which provide some F_0) to increase the level of sterility confidence should be considered.

A list of references, which may be of value to the reader, is included at the conclusion of this document.

IV. BUILDINGS AND FACILITIES

> Section 211.42 (design and construction features) requires, in part, that aseptic processing operations be "performed within specifically defined areas of adequate size. There shall be separate or defined areas for the firm's operations to prevent contamination or mixups." Aseptic processing operations must also "include, as appropriate, an air supply filtered through high efficiency particulate air (HEPA) filters under positive pressure," as well as systems for "monitoring environmental conditions..."and "maintaining any equipment used to control aseptic conditions."
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> Section 211.46 (ventilation, air filtration, air heating and cooling) states, in part, that "equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing or holding of a drug product." This regulation also states that "air filtration systems, including pre-filters and particulate matter air filters, shall be used when appropriate on air supplies to production areas."

In aseptic processing, there are various areas of operation which require separation and control, with each area having different degrees of air quality depending on the nature of the operation. Area design is based upon satisfying microbiological and particulate standards defined by the equipment, components, and products exposed, as well as the particular operation conducted, in the given area.

Critical and support areas of the aseptic processing operation should be classified and supported by microbiological and particulate data obtained during qualification studies. While initial clean room qualification includes some assessment of air quality under as-built and static conditions,

the final room or area classification should be derived from data generated under dynamic conditions, i.e., with personnel present, equipment in place, and operations ongoing. The aseptic processing facility monitoring program should assess conformance with specified clean area classifications under dynamic conditions, on a routine basis.

The following table summarizes clean area air classifications (Ref. 1).

TABLE 1- Air Classifications[a]

Clean Area Classification	≥ 0.5 um particles/ft^3	≥ 0.5 um particles/m^3	Microbiological Limit[b]	
			cfu/10 ft^3	cfu/m^3
100	100	3,500	<1[c]	<3[c]
1000	1000	35,000	≤ 2	≤7
10,000	10,000	350,000	≤ 5	≤ 18
100,000	100,000	3,500,000	≤25	≤ 88

a- All classifications based on data measured in the vicinity of exposed articles during periods of activity.
b- Alternate microbiological standards may be established where justified by the nature of the operation. c- Samples from class 100 environments should normally yield no microbiological contaminants.

Two clean areas are of particular importance to sterile drug product quality: the critical area and the supporting clean areas associated with it.

A. Critical Area (Class 100)

A critical area is one in which the sterilized drug product, containers, and closures are exposed to environmental conditions designed to preserve sterility. Activities conducted in this area include manipulations (e.g., aseptic connections, sterile ingredient additions) of sterile materials prior to and during filling and closing operations.

This area is critical because the product is not processed further in its immediate container and is vulnerable to contamination. In order to maintain product sterility, the environment in which aseptic operations are conducted should be of appropriate quality throughout operations. One aspect of environmental quality is the particulate content of the air. Particulates are significant because they can enter a product and contaminate it physically or, by acting as a vehicle for microorganisms, biologically. Particle content in critical areas should be minimized by effective air systems.

Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations is of acceptable particulate quality when it has a per-cubic-foot particle count of no more than 100 in a size range of 0.5 micron and larger (Class 100) when counted at representative locations normally not more than one foot away from the work site, within the airflow, and during filling/closing operations. Deviations from this critical area monitoring parameter should be documented as to origin and significance.

Measurements to confirm air cleanliness in aseptic processing zones should be taken with the particle counting probe oriented in the direction of oncoming airflow and at specified sites where sterilized product and container-closure are exposed. Regular monitoring should be performed during each shift. Nonviable particulate monitoring with a remote counting system is generally less invasive than the use of portable particle counting units and provides the most comprehensive data. See Section X.D, "Particulate Monitoring."

Some powder filling operations can generate high levels of powder particulates that, by their nature, do not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one foot distance and still differentiate "background noise" levels of powder particles from air contaminants. In these instances, air should be sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particulate contamination to which the product is exposed. Initial certification of the area under dynamic conditions without the actual powder filling function should provide some baseline information on the non-product particle generation of the operation.

Air in critical areas should be supplied at the point of use as HEPA filtered laminar flow air at a velocity sufficient to sweep particulate matter away from the filling/closing area and maintain laminarity during operations. The velocity parameters established for each processing line should be justified, and appropriate to maintain laminarity and air quality under dynamic conditions within a defined space (Ref. 2).[3]

Proper design and control should prevent turbulence or stagnant air in the aseptic processing line or clean zone. Once relevant parameters are established, airflow patterns should be evaluated for turbulence. Air pattern or "smoke" studies demonstrating laminarity and sweeping action over and away from the product under dynamic conditions should be conducted. The studies should be well-documented with written conclusions. Videotape or other recording mechanisms have been found to be useful in assessing airflow initially as well as facilitating evaluation of subsequent equipment configuration changes. However, even successfully qualified systems can be compromised by poor personnel, operational, or maintenance practices.

Active air monitoring of critical areas should normally yield no microbiological contaminants. Contamination in this environment should receive investigative attention.

B. Supporting Clean Areas

Supporting clean areas include various classifications and functions. Many support areas function as zones in which non-sterile components, formulated product, in-process materials, equipment, and container/closures are prepared, held, or transferred. These environments should be designed to minimize the level of particulate contaminants in the final product and control the microbiological content (bioburden) of articles and components that are subsequently sterilized.

The nature of the activities conducted in a supporting clean area should determine its classification. An area classified at Class 100,000 would be used for less critical activities (such

[3] A velocity from 90 to 100 feet per minute is generally established, with a range of plus or minus 20% around the setpoint. Higher velocities may be appropriate in operations generating high levels of particulates.

as initial equipment preparation). The area immediately adjacent to the aseptic processing line should, at a minimum, meet Class 10,000 standards (see Table 1) under dynamic conditions. Depending on the operation, manufacturers can also classify this area as Class 1000 or maintain the entire aseptic filling room at Class 100.

C. Clean Area Separation

Adequate separation is necessary between areas of operation to prevent contamination (211.42). In order to maintain air quality in areas of higher cleanliness, it is important to achieve a proper airflow and a positive pressure differential relative to adjacent less clean areas. Rooms of higher classification should have a positive pressure differential relative to adjacent lower classified areas of generally at least 0.05 inch of water (with doors closed). When doors are open, outward airflow should be sufficient to minimize ingress of contamination (Ref. 3). Pressure differentials between clean rooms should be monitored continuously throughout each shift and frequently recorded, and deviations from established limits investigated.

An adequate air change rate should be established for a cleanroom. For Class 100,000 supporting rooms, airflow sufficient to achieve at least 20 air changes per hour is typically acceptable.

Facility monitoring systems should be established to rapidly detect atypical changes that can compromise the facility's environment. Operating conditions should be restored to established, qualified levels before reaching action levels. For example, pressure differential specifications should enable prompt detection (i.e., alarms) of any emerging low pressure problem in order to preclude ingress of unclassified air into a classified room.

D. Air Filtration

1. Membrane (Compressed Gases)

A compressed gas should be of appropriate purity (e.g., free from oil and water vapor) and its microbiological and particulate quality should be equal to or better than air in the environment into which the gas is introduced. Compressed gases such as air, nitrogen, and carbon dioxide are often used in clean rooms and are frequently employed in operations involving purging or overlaying.

Membrane filters allow for the filtration of compressed gases to meet an appropriate high quality standard, and can be used to produce a sterile compressed gas. A sterile-filtered gas is used when the gas contacts a sterilized material. Certain equipment also should be supplied with a sterile-filtered gas. For example, sterile bacterial retentive membrane filters should be used for autoclave air lines, lyophilizer vacuum breaks, vessels containing sterilized materials, and hot air sterilizer vents. Sterilized tanks or liquids should be held under continuous overpressure to prevent microbial contamination. Safeguards should be in place to prevent a pressure change that can result in contamination due to back flow of non-sterile air or liquid.

Gas filters (including vent filters) should be dry. Condensate in a gas filter can cause blockage or microbial contamination. Frequent replacement, heating, and use of hydrophobic filters prevent moisture residues in a gas supply system. These filters also should be integrity tested upon installation, and periodically thereafter (e.g., including at end of use). Integrity test failures should be investigated.

2. *High Efficiency Particulate Air (HEPA)*[4]

An essential element in ensuring aseptic conditions is the maintenance of HEPA filter integrity. Integrity testing should be performed at installation to detect leaks around the sealing gaskets, through the frames or through various points on the filter media. Thereafter, integrity tests should be performed at suitable time intervals for HEPA filters in the aseptic processing facility. For example, such testing should be performed twice a year for the aseptic processing room. Additional testing may be needed when air quality is found to be unacceptable, or as part of an investigation into a media fill or drug product sterility failure. Among the filters that should be integrity tested are those installed in dry heat depyrogenation tunnels commonly used to depyrogenate glass vials.

One recognized method of testing the integrity of HEPA filters is use of a dioctylphthalate (DOP) aerosol challenge. However, alternative aerosols may be acceptable. Poly-alpha-olefin can also be used, provided it meets specifications for critical physicochemical attributes such as viscosity. Some alternative aerosols are problematic because they pose a risk of microbial contamination of the environment being tested. Firms should ensure that any alternative does not promote microbial growth.

An intact HEPA filter is capable of retaining at least 99.97 percent of particulates greater than 0.3 micron in diameter. It is important to ensure that the aerosol used for the challenge has a sufficient number of particles of this size range. Performing an integrity test without introducing particles of known size upstream of the filter is ineffective for detecting leaks. The DOP challenge should introduce the aerosol upstream of the filter in a concentration of 80 to 100 micrograms/liter of air at the filter's designed airflow rating. The downstream side of the filter is then scanned with an appropriate photometer probe at a sampling rate of at least one cubic foot per minute. Scanning should be conducted on the entire filter face and frame at a position about one to two inches from the face of the filter. This comprehensive scanning of HEPA filters should be fully documented. While vendors often provide these services, the drug manufacturer is responsible for ensuring that these essential certification activities are conducted satisfactorily. A single probe reading equivalent to 0.01 percent of the upstream challenge should be considered as indicative of a significant leak and should result in replacement of the HEPA filter or perhaps repair in a limited area. A subsequent confirmatory re-test should be performed in the area of any repair.

There is a major difference between filter integrity testing and efficiency testing. The purpose of regularly scheduled integrity testing is to detect leaks from the filter media, filter frame and seal. The challenge is a polydispersed aerosol usually composed of particles ranging in size from one

[4] The same broad principles can be applied to ULPA filters.

to three microns. The test is done in place and the filter face is scanned with a probe; the measured downstream leakage is taken as a percent of the upstream challenge. The efficiency test, on the other hand, is a test used only to determine the rating of the filter.[5]

HEPA filter integrity testing alone is not sufficient to monitor filter performance. This testing is usually done only on a semi-annual basis. It is important to conduct periodic monitoring of filter attributes such as uniformity of velocity across the filter (and relative to adjacent filters). Variations in velocity generally increase the possibility of contamination, as these changes (e.g., velocity reduction) can have an effect on the laminarity of the airflow. Airflow velocities are measured six inches from the filter face or at a defined distance proximal to the work surface for each HEPA filter. For example, velocity monitoring as frequently as weekly may be appropriate for the clean zone in which aseptic processing is performed. HEPA filters should be replaced when inadequate airflow (e.g., due to blockage) or non-uniformity of air velocity across an area of the filter is detected.

E. Design

Section 211.42 requires that aseptic processing operations be "performed within specifically defined areas of adequate size. There shall be separate or defined areas for the firm's operations to prevent contamination or mixups."

Section 211.42 states that "flow of components, drug products containers, closures, labeling, in-process materials, and drug products through the building or building shall be designed to prevent contamination." HEPA filtered air as appropriate, as well as "floors, walls and ceilings of smooth, hard surfaces that are easily cleanable" are some additional requirements of this section.

Section 211.63 states that equipment "shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

Section 211.65 states that "equipment shall be constructed so that surfaces that contact the components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

Section 211.68 includes requirements for "automatic, mechanical and electronic equipment."

Section 211.113 states that "appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed."

An aseptic process is designed to minimize exposure of sterile articles to dynamic conditions and potential contamination hazards presented by the operation. Limiting the duration of open container exposure, providing the highest possible environmental control, and designing equipment to prevent entrainment of lower quality air into the Class 100 zone are essential to this goal (Ref. 3).

[5] The efficiency test uses a monodispersed aerosol of 0.3 micron size particles, relates to filter media, and usually requires specialized testing equipment. Downstream readings represent an average over the entire filter surface. Therefore, the efficiency test is not intended to test for leakage in a filter.

Any intervention or stoppage during an aseptic process can increase the risk of contamination. Personnel and material flow should be optimized to prevent unnecessary activities that increase the potential for introducing contaminants to exposed product, container-closures, or the surrounding environment. The layout of equipment should provide for ergonomics that optimize comfort and movement of operators. The flow of personnel should be designed to limit the frequency with which entries and exits are made to and from the aseptic processing room and, more significantly, its critical area. In order to prevent changes in air currents that introduce lower quality air, movement adjacent to the critical area should be limited. For example, personnel intervention can be reduced by integrating an on-line weight check device, thus eliminating a repeated manual activity within the critical zone. It is also important to minimize the number of personnel in the aseptic processing room.

Transfer of products should be performed under appropriate clean room conditions. For example, lyophilization processes include transfer of aseptically filled product in partially-sealed containers. To prevent contamination, partially-closed sterile product should be staged and transferred only in critical areas. Facility design should assure that the area between a filling line and the lyophilizer, and the transport and loading procedures, provide Class 100 protection.

The sterile product and container-closures should also be protected from activities occurring adjacent to the line. Carefully designed curtains, rigid plastic shields, or other barriers should be used in appropriate locations to partially segregate the aseptic processing line.

Airlocks and interlocking doors facilitate better control of air balance throughout the aseptic processing area. Airlocks should be installed between the aseptic processing area entrance and the adjoining uncontrolled area. Other interfaces such as personnel entries, or the juncture of the aseptic processing room and its adjacent room, are also appropriate locations for air locks.

Clean rooms are normally designed as functional units with specific purposes. A well-designed clean room is constructed with material that allows for ease of cleaning and sanitizing. Examples of adequate design features include seamless and rounded floor to wall junctions as well as readily accessible corners. Floors, walls, and ceilings are constructed of smooth, hard surfaces that can be easily cleaned (211.42). Ceilings and associated HEPA filter banks should be designed to protect sterile materials from contamination. Clean rooms also should not contain unnecessary equipment, fixtures, or materials.

Processing equipment and systems should be equipped with sanitary fittings and valves. Drains are not considered appropriate for rooms in classified areas of the aseptic processing facility.

When applicable, equipment must be suitably designed for ease of sterilization (211.63). The effect of equipment layout and design on the clean room environment should be addressed. Flat surfaces or ledges that accumulate dust and debris should be avoided. Equipment should not obstruct airflow and, in critical zones, its design should not perturb airflow.

V. PERSONNEL TRAINING, QUALIFICATION, & MONITORING

Sections 211.22 states that "the quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product."

Section 211.113(b) addresses the procedures designed to prevent microbiological contamination, stating that "appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed."

Section 211.25, Personnel Qualifications requires that "each person engaged in manufacture, processing, packing or holding of a drug product shall have education, training and experience, or any combination thereof, to enable that person to perform the assigned functions...Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess." This section also requires "an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing or holding of each drug product." Section 211.25 also requires that continuing training in CGMP "shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them." The training "shall be in the particular operations that the employee performs and in current good manufacturing practice (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations), as they relate to the employee's functions."

Section 211.28, Personnel Responsibilities states, that "personnel engaged in the manufacture, processing, packing or holding of a drug product shall wear clean clothing appropriate for the duties they perform." It also states that "personnel shall practice good sanitization and health habits" and specifies that "protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination." It also states that "any person shown at any time (either by medical examination or supervisory examination) to have an apparent illness or open lesions that may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardize the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products."

This section also addresses restrictions on entry into limited access areas: "Only personnel authorized by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas."

Section 211.42 requires the establishment of a "system for monitoring environmental conditions."

A. Manufacturing Personnel

A well-designed aseptic process minimizes personnel intervention. As operator activities increase in an aseptic processing operation, the risk to finished product sterility also increases. It is essential that operators involved in aseptic manipulations adhere to the basic principles of aseptic technique at all times to assure maintenance of product sterility.

Appropriate training should be conducted before an individual is permitted to enter the aseptic processing area and perform operations. For example, such training should include aseptic technique, clean room behavior, microbiology, hygiene, gowning, and patient safety hazard posed by a non-sterile drug product, and the specific written procedures covering aseptic processing area operations. After initial training, personnel should be updated regularly by an ongoing training program. Supervisory personnel should routinely evaluate each operator's conformance to written procedures during actual operations. Similarly, the quality control unit

should provide regular oversight of adherence to established, written procedures and basic aseptic techniques during manufacturing operations.

Adherence to basic aseptic technique is a continuous requirement for operators in an aseptic processing operation. Some of these techniques aimed at maintaining sterility of sterile items and surfaces include:

1. *Contacting sterile materials only with sterile instruments.* Sterile instruments (e.g., forceps) are should always be used in the handling of sterilized materials. Between uses, instruments should be placed only in sterilized containers. These instruments should be replaced as necessary throughout the operation.

   After initial gowning, sterile gloves should be regularly sanitized to minimize the risk of contamination. Personnel should not directly contact sterile products, containers, closures, or critical surfaces.

2. *Moving slowly and deliberately.* Rapid movements can create unacceptable turbulence in the critical zone. Such movements disrupt the sterile field, presenting a challenge beyond intended cleanroom design and control parameters. The principle of slow, careful movement should be followed throughout the cleanroom.

3. *Keeping the entire body out of the path of laminar air.* Laminar airflow design is used to protect sterile equipment surfaces, container-closures, and product. Personnel should not disrupt the path of laminar flow air in the aseptic processing zone.

4. *Approaching a necessary manipulation in a manner that does not compromise sterility of the product.* In order to maintain sterility of nearby sterile materials, a proper aseptic manipulation should be approached from the side and not above the product (in vertical laminar flow operations). Also, speaking when in direct proximity to an aseptic processing line is not an acceptable practice.

Personnel who have been qualified and permitted access to the aseptic processing area should be appropriately gowned. An aseptic processing area gown should provide a barrier between the body and exposed sterilized materials, and prevent contamination from particles generated by, and microorganisms shed from, the body. Gowns need to be sterile and non-shedding, and should cover the skin and hair. Face masks, hoods, beard/moustache covers, protective goggles, elastic gloves, clean room boots, and shoe overcovers are examples of common elements of gowns. An adequate barrier should be created by the overlapping of gown components (e.g., gloves overlapping sleeves). If an element of the gown is found to be torn or defective, it should be changed immediately.

There should be an established program to regularly assess or audit conformance of personnel to relevant aseptic manufacturing requirements. An aseptic gowning qualification program should assess the ability of a cleanroom operator to maintain the sterile quality of the gown after performance of gowning procedures. Gowning qualification should include microbiological surface sampling of several locations on a gown (e.g., glove fingers, facemask, forearm, chest, other sites). Following an initial assessment of gowning, periodic requalification should monitor

various gowning locations over a suitable period to ensure the consistent acceptability of aseptic gowning techniques. Semi-annual or yearly requalification is acceptable for automated operations where personnel involvement is minimized.

To protect exposed sterilized product, personnel are expected to maintain sterile gown quality and aseptic method standards in a consistent manner. Written procedures should adequately address circumstances under which personnel should be retrained, requalified, or reassigned to other areas.

B. Laboratory Personnel

The basic principles of training, aseptic technique, and personnel qualification in aseptic manufacturing are equally applicable to those performing aseptic sampling and microbiological laboratory analyses. Processes and systems cannot be considered to be in control and reproducible if there is any question regarding the validity of data produced by the laboratory.

C. Monitoring Program

Personnel can have substantial impact on the quality of the environment in which the sterile product is processed. A vigilant and responsive personnel monitoring program should be established. Monitoring should be accomplished by obtaining surface samples of each aseptic processing operator's gloves on an at least a daily basis, or in association with each batch. This sampling should be accompanied by an appropriate sampling frequency for other strategically selected locations of the gown (Ref. 7). The quality control unit should establish a more comprehensive monitoring program for operators involved in operations which are especially labor intensive, i.e. those requiring repeated or complex aseptic manipulations.

Asepsis is fundamental to an aseptic processing operation. An ongoing goal for manufacturing personnel in the aseptic processing room is to maintain contamination-free gloves throughout operations. Sanitizing gloves just prior to sampling is inappropriate because it can prevent recovery of microorganisms that were present during an aseptic manipulation. When operators exceed established levels or show an adverse trend, an investigation should be conducted promptly. Follow-up actions may include increased sampling, increased observation, retraining, gowning requalification, and in certain instances, reassignment of the individual to operations outside of the aseptic processing area. Microbiological trending systems, and assessment of the impact of atypical trends, are discussed in more detail under Section XI., Laboratory Controls.

VI. COMPONENTS AND CONTAINER/CLOSURES

A. Components

Section 210.3(b)(3) defines a "component" as "any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product."

Section 211.80, General Requirements, requires, in part, the establishment of written procedures "describing in sufficient detail the receipt, identification, storage, handling, sampling, sampling, testing, and approval or rejection of components and drug product containers and closures...Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination."

Section 211.84, Testing and approval or rejection of components, drug product containers, and closures, requires that "each lot of a component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use."

A drug product produced by aseptic processing can become contaminated by use of one or more components (e.g., active ingredients, excipients, Water for Injection) that are contaminated with microorganisms or endotoxins. It is important to characterize the microbial content of each component liable to contamination and establish appropriate acceptance/rejection limits based on information on bioburden. Knowledge of bioburden is critical in assessing whether the sterilization process is adequate.

In aseptic processing, each component is individually sterilized or several components are combined, with the resulting mixture sterilized.[6] There are several methods to sterilize components (see relevant discussion in Section IX). A widely used method is filtration of a solution formed by dissolving the component(s) in a solvent such as USP Water For Injection (WFI). The solution is passed through a sterilizing membrane or cartridge filter. Filter sterilization is used where the component is soluble and is likely to be adversely affected by heat. A variation of this method involves subjecting the filtered solution to aseptic crystallization and precipitation of the component as a sterile powder. However, this method involves more handling and manipulation and therefore has a higher potential for contamination during processing. If a component is not adversely affected by heat, and is soluble, it may be made into a solution and subjected to steam sterilization, typically in an autoclave or a pressurized vessel.

Dry heat sterilization is a suitable method for components that are heat stable and insoluble. However, carefully designed heat penetration and distribution studies should be performed for powder sterilization because of the insulating effects of the powder.

Ethylene oxide (EtO) exposure is often used for surface sterilization. Such methods should be carefully controlled and validated if used for powders to evaluate whether consistent penetration of the sterilant is achieved and to minimize residual ethylene oxide and by-products.

Parenteral products are intended to be non-pyrogenic. There should be written procedures and appropriate specifications for acceptance or rejection of each lot of components that might contain endotoxins. Any components failing to meet endotoxin specifications should be rejected.

B. Containers/Closures

[6] See Appendix III for discussion of certain biologic components that are aseptically handled from the start of the process.

Section 211.94 (drug product containers and closures) states that "drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use." It also states that "Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing and processing to remove pyrogenic properties shall be written and followed for drug product containers and closures."

Section 211.113(b) requires "validation of any sterilization process" as part of designing procedures "to prevent microbiological contamination of drug products purporting to be sterile."

1. Preparation

Containers and closures should be rendered sterile and, for parenteral drug products, pyrogen-free. The type of processes used will depend primarily on the nature of the material comprising the container and/or closure. The validation study for any such process should be adequate to demonstrate its ability to render materials sterile and pyrogen-free. Written procedures should specify the frequency of revalidation of these processes as well as time limits for holding sterile, depyrogenated containers and closures.

Presterilization preparation of glass containers usually involves a series of wash and rinse cycles. These cycles serve an important role in removing foreign matter. Rinse water should be of high purity so as not to contaminate containers. For parenteral products, final rinse water should meet the specifications of Water for Injection, USP.

The adequacy of the depyrogenation process can be assessed by spiking containers or closures with known quantities of endotoxin, followed by measuring endotoxin content after depyrogenation. The challenge studies should be performed with a reconstituted endotoxin solution applied directly onto the surface being tested and air-dried. Positive controls should be used to measure the percentage of endotoxin recovery by the test method. Validation study data should demonstrate that the process reduces the endotoxin content by at least 99.9% (3 logs).

Glass containers are generally subjected to dry heat for sterilization and depyrogenation. Validation of dry heat sterilization/depyrogenation should include appropriate heat distribution and penetration studies as well as the use of worst-case process cycles, container characteristics (e.g., mass), and specific loading configurations to represent actual production runs. See Section IX.C.

Pyrogen on plastic containers can be generally removed by multiple WFI rinses. Plastic containers can be sterilized with an appropriate gas, irradiation or other suitable means. For gases such as EtO, the parameters and limits of the EtO sterilization cycle (e.g. temperature, pressure, humidity, gas concentration, exposure time, degassing, aeration, and determination of residuals) should be specified and monitored closely. Biological indicators are of special importance in demonstrating the effectiveness of EtO and other gas sterilization processes.

Rubber closures (e.g., stoppers and syringe plungers) are cleaned by multiple cycles of washing and rinsing prior to final steam or irradiation sterilization. At minimum, the initial rinses for the washing process should employ Purified Water USP of minimal endotoxin content, followed by final rinse(s) with WFI for parenteral products. Normally, depyrogenation is achieved by

multiple rinses of hot WFI. The time between washing and sterilizing should be minimized because moisture on the stoppers can support microbial growth and the generation of endotoxins. Because rubber is a poor conductor of heat, extra attention should be given to the validation of processes that use heat to sterilize rubber stoppers. Validation data should also demonstrate successful endotoxin removal from rubber materials.

A potential source of contamination is the siliconization of rubber stoppers. Silicone used in the preparation of rubber stoppers should be rendered sterile and should not have an adverse effect on the safety, quality, or purity of the drug product.

See Section VIII for discussion of the need to establish production time limits for the holding of sterilized containers and closures.

Contract facilities that perform sterilization and depyrogenation of containers and closures are subject to the same CGMP requirements as those established for in-house processing. The finished dosage form manufacturer is responsible for the review and approval of the contractor's validation protocol and final validation report.

2. Inspection of Container-Closure System

A container-closure system that permits penetration of air, or microorganisms, is unsuitable for a sterile product. Any damaged or defective units should be detected, and removed, during inspection of the final sealed product. Safeguards should be implemented to strictly preclude shipment of product that may lack container-closure integrity and lead to non-sterility. Equipment suitability problems or incoming container or closure deficiencies have caused loss of container-closure system integrity. As examples, failure to detect vials fractured by faulty machinery, or by mishandling of bulk finished stock, has led to drug recalls. If damage that is not readily detected leads to loss of container-closure integrity, improved procedures should be rapidly implemented to prevent and detect such defects.

Functional defects in delivery devices (e.g., syringe device defects, delivery volume) can also result in product quality problems, and should be monitored by appropriate in-process testing.

Any defects or results outside the specifications established for in-process and final inspection should be investigated in accord with Section 211.192.

VII. ENDOTOXIN CONTROL

Section 211.63, equipment design, size, and location, states that equipment "shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance."

Section 211.65, equipment construction requires, in part, that "equipment shall be constructed so that surfaces that contact the components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality or purity of the drug product beyond the official or other established requirements."

Section 211.67, equipment cleaning and maintenance requires, states that "equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety,

identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

Section 211.94 states that "drug product containers and closures shall be clean, and where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use."

Section 211.167 states: "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

Endotoxin contamination of an injectable product can be a result of poor CGMP controls. Certain patient populations (e.g., neonates), those receiving other injections concomitantly, or those administered a parenteral in atypically large volumes or doses can be at greater risk for pyrogenic reaction than anticipated by the established limits based on body weight of a normal healthy adult (Ref. 6,7). Such clinical concerns reinforce the need for appropriate CGMP controls to prevent generation of endotoxin. Drug product components, container-closures, equipment, and storage time limitations are among the concerns to address in establishing endotoxin control.

Adequate cleaning, drying, and storage of equipment provides for control of bioburden and prevents contribution of endotoxin load. Equipment should be designed to be easily assembled and disassembled, cleaned, sanitized, and/or sterilized. Endotoxin control should be exercised for all product contact surfaces both prior to and after sterile filtration.

Endotoxin on equipment surfaces is inactivated by high temperature dry heat, or removed from equipment surfaces by validated cleaning procedures. Some clean-in-place procedures employ initial rinses with appropriate high purity water and/or a cleaning agent (e.g., acid, base, surfactant), followed by final rinses with heated WFI. Equipment should be dried following cleaning. Sterilizing filters and moist heat sterilization have not been shown to be effective in removing endotoxins. Processes that are designed to achieve depyrogenation should demonstrate a 3-log reduction of endotoxin.

VIII. TIME LIMITATIONS

Section 211.111 (time limitations on production) states: "When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product."

Time limits should be established for each phase of aseptic processing. Time limits should include, for example, the period between the start of bulk product compounding and its filtration, filtration processes, product exposure while on the processing line, and storage of sterilized equipment, containers and closures. Maintenance of in-process quality at different production phases should be supported by data. Bioburden and endotoxin load should be assessed when establishing time limits for stages such as the formulation processing stage.

The total time for product filtration should be limited to an established maximum in order to prevent microorganisms from penetrating the filter. Such a time limit should also prevent a

significant increase in upstream bioburden and endotoxin load. Sterilizing filters should generally be replaced following each manufactured lot. Because they can provide a substrate for microbial attachment, maximum use times for those filters used upstream for solution clarification or particle removal should also be established and justified.

IX. PROCESS VALIDATION AND EQUIPMENT QUALIFICATION

> Section 211.113(b) (control of microbiological contamination) states: "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process."
>
> Sections 211.63, 211.65, and 211.67 address, respectively, "Equipment, design, size, and location," "Equipment construction," and "Equipment cleaning and maintenance."
>
> Section 211.84(c)(3) states that "sterile equipment and aseptic sampling techniques shall be used when necessary."

The following sections primarily discuss routine qualification and validation study expectations. Change control procedures are only briefly addressed, but are an important part of the quality systems established by a firm. A change in equipment, process, test method, or systems requires evaluation through the written change control program, and should trigger an evaluation of the need for revalidation or requalification.

A. Process Simulations

In order to ensure the sterility of products purporting to be sterile, both sterilization and aseptic filling/closing operations must be adequately validated (211.113). The goal of even the most effective sterilization processes can be defeated if the sterilized elements of a product (the drug, the container and the closure) are brought together under conditions that contaminate those elements. Similarly, product sterility is compromised when the product elements are non-sterile at the time they are assembled.

The validation of an aseptic processing operation should include the use of a microbiological growth nutrient medium in place of product. This has been termed a "media fill" or "process simulation." The nutrient medium is exposed to product contact surfaces of equipment, container systems, critical environments, and process manipulations to closely simulate the same exposure that the product itself will undergo. The sealed containers filled with the media are then incubated to detect microbial contamination. The results are interpreted to determine the potential for any given unit of drug product to become contaminated during actual operations (e.g., start-up, sterile ingredient additions, aseptic connections, filling, closing). Environmental monitoring data is integral to the validation of an aseptic processing operation.

1. Study Design

A validation protocol should detail the overall strategy, testing requirements, and acceptance criteria for the media fill. Media fill studies should simulate aseptic manufacturing operations as

closely as possible, incorporating a "worst-case" approach. A media fill study should address applicable issues such as:

a) factors associated with the longest permitted run on the processing line
b) ability to produce sterile units when environmental conditions impart a greater risk to the product.
c) number and type of normal interventions, atypical interventions, unexpected events (e.g., maintenance), stoppages, equipment adjustments or transfers
d) lyophilization, when applicable
e) aseptic assembly of equipment (e.g., at start-up, during processing)
f) number of personnel and their activities
g) number of aseptic additions (e.g., charging containers and closures as well as sterile ingredients)
h) shift changes, breaks, and gown changes (when applicable)
i) number and type of aseptic equipment disconnections/connections
j) aseptic sample collections
k) line speed and configurations
l) manual weight checks
m) operator fatigue
n) container-closure systems (e.g., sizes, type, compatibility with equipment)
o) consideration of temperature and humidity set point extremes
p) specific provisions of aseptic processing related Standard Operating Procedures (conditions permitted before line clearance is mandated, etc.).

A written batch record, documenting conditions and activity simulated, should be prepared for each media fill run. The same vigilance should be observed in both media fill and routine production runs. Media fills cannot be used to "validate" an unacceptable practice.

2. Frequency and number of runs

When a processing line is initially validated, separate media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control. A minimum of three consecutive separate successful runs should be performed during initial line qualification. Subsequently, routine semi-annual revalidation runs should be conducted for each shift and processing line to evaluate the state of control of the aseptic process. All personnel who enter the aseptic processing area, including technicians and maintenance personnel, should participate in a media fill at least once a year

Each change to a product or line change should be evaluated using a written change control system. Any changes or events that appear to affect the ability of the aseptic process to exclude contamination from the sterilized product should be assessed through additional media fills. For example, facility and equipment modification, line configuration change, significant changes in personnel, anomalies in environmental testing results, container-closure system changes or, end product sterility testing showing contaminated products may be cause for revalidation of the system.

Where a media fill's data indicates the process may not be in control, a comprehensive documented investigation should be conducted to determine the origin of the contamination and the scope of the problem. Once corrections are instituted, multiple repeat process simulation runs should be performed to confirm that deficiencies in practices and procedures have been corrected and the process has returned to a state of control. However, when an investigation fails to reach well-supported, substantive conclusions as to the cause of the media fill failure, three consecutive successful runs and increased scrutiny (i.e., extra supervision, monitoring) of the production process should be implemented.

3. Size and Duration of runs

The duration of aseptic processing operations is a major consideration in determining the size of the media fill run. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production run, other appropriate models can be justified. In any study protocol, the duration of the run and the overall study design should adequately mimic worst-case operating conditions and cover all manipulations that are performed in the actual processing operation. Adequate batch sizes are needed to simulate commercial production conditions and accurately assess the potential for commercial batch contamination. The number of units filled should be sufficient to reflect the effects of potential operator fatigue, as well as the maximum number of interventions and stoppages. The run should be large enough to accurately simulate production conditions and sensitive enough to detect a low incidence of contaminated units. For batches produced over multiple shifts or yielding an unusually large number of units, the media fill protocol should adequately encompass conditions and any potential risks associated with the larger operation.

While conventional manufacturing lines are highly automated, often operate at relatively high speeds, and are designed to limit operator intervention, there are some processes that include considerable operator involvement. When aseptic processing employs manual filling or closing, or extensive manual manipulations, the duration of the process simulation should generally be no less than the length of the actual manufacturing process in order to best simulate operator fatigue.

For simulation of lyophilization operations, unsealed containers should be exposed to pressurization and partial evacuation of the chamber in a manner that is representative of process stresses. Vials should not be frozen, as this may inhibit the growth of microorganisms.

4. Line Speed

The media fill program should adequately address the range of line speeds (e.g., by bracketing all vial sizes and fill volumes) employed during production. In some cases, more than one line speed should be evaluated in the course of a study.

Each individual media fill run should evaluate a single worst-case line speed and the speed chosen for each batch during a study should be justified. For example, use of high line speed is justified for manufacturing processes characterized by frequent interventions or a significant

degree of manual manipulation. Use of slow line speed is justified for manufacturing processes characterized by prolonged exposure of sterile components in the aseptic area

5. Environmental Conditions

Media fills should be conducted under environmental conditions that simulate normal as well as "worst case" conditions of production. An inaccurate assessment (making the process appear "cleaner" than it actually is) can result from conducting a media fill under extraordinary air particulate and microbial quality, or under production controls and precautions taken in preparation for the media fill. To the extent standard operating procedures permit stressful conditions, it is crucial that media fills should include rigorous challenges in order to support the validity of these studies.

6. Media

In general, a microbiological growth medium such as soybean casein digest medium should be used. Use of anaerobic growth media (such as Fluid Thioglycollate Medium) is appropriate in special circumstances. Media selected should be demonstrated to promote growth of USP <71> indicator microorganisms as well as isolates that have been identified by environmental monitoring, personnel monitoring, and positive sterility test results. Positive control units should be inoculated with a <100 CFU challenge and incubated. For those instances in which the growth promotion testing fails, the origin of any contamination found during the simulation should nonetheless be investigated and the media fill should be promptly repeated.

The production process should be accurately simulated using media and conditions that optimize detection of any microbiological contamination. Each unit should be filled with an appropriate quantity and type of microbial growth medium to contact the inner container-closure surfaces (when the unit is inverted and swirled) and permit visual detection of microbial growth.

Some drug manufacturers have expressed concern over the possible contamination of the facility and equipment with the nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

7. Incubation and Examination of Media Filled Units

Media units should be incubated for a sufficient time (a period of not less than 14 days) at a temperature adequate to enhance detection of organisms that can otherwise be difficult to culture.

Each media filled unit should be examined for contamination by personnel with appropriate education, training and experience in microbiological techniques. There should be direct quality control unit oversight throughout any such examination. Clear containers with otherwise identical physical properties should be used as a substitute for amber or other opaque containers to allow visual detection of microbial growth.

When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. Units found to have defects not related to integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected.[7] Erroneously rejected units should be returned promptly for incubation with the media fill lot.

After incubation is underway, any unit found to be damaged should be included in the data for the media fill batch, because the incubation of the units simulates release to the market. Any decision to exclude such incubated units (i.e., non-integral) from the final batch tally should be fully justified, and the deviation explained in the media fill report. If a correlation emerges between difficult to detect damage and microbial contamination, a thorough investigation should be conducted to determine its cause (See Section VI.B).

Written procedures regarding aseptic interventions should be clear and specific (e.g., intervention type; quantity of units removed), providing for consistent production practices and assessment of these practices during media fills. If written procedures and batch documentation are adequate, these intervention units do not need to be incubated during media fills. Where procedures lack specificity, there would be insufficient justification for exclusion of units removed during an intervention from incubation. As an example, if a production procedure requires removal of ten units after an intervention at the stoppering station infeed, batch records (i.e., for production and media fills) should clearly document conformance with this procedure. In no case should more units be removed during a media fill intervention than would be cleared during a production run. The ability of a media fill run to detect potential contamination from a given simulated activity should not be compromised by a large scale line clearance, which can result in removal of a positive unit caused by an unrelated event or intervention. If unavoidable, appropriate study provisions should be made to compensate in such instances.

Appropriate criteria should be established for yield and accountability. Batch record reconciliation documentation should include an accurate accounting and description of units rejected from a batch.

8. Interpretation of Test Results

The process simulation run should be observed, and contaminated units should be reconcilable with the approximate time and the activity being simulated during the media fill. Videotaping of a media fill has been found to be useful in identifying personnel practices which could negatively impact on the aseptic process.

Any contaminated unit should be considered as objectionable and fully investigated. The microorganisms should be identified to species level. In the case of a media fill failure, a comprehensive investigation should be conducted, surveying all possible causes of the contamination. The impact on commercial drugs produced on the line since the last successful media fill should also be assessed.

[7] Separate incubation of certain categories of rejected units may nonetheless provide valuable information with respect to contamination that may arise from container/closure integrity deficiencies.

Whenever contamination exists in a media fill batch, it should be considered as indicative of a potential production problem. The use of statistics has limitations for media fill evaluation in that the number of contaminated units should not be expected to increase in a directly proportional manner with the number of vials in the media fill run. Test results should show, with a high degree of confidence, that the units produced by an aseptic processing operation are sterile. Modern aseptic processing operations in suitably designed facilities have demonstrated a capability of meeting contamination levels approaching zero (Ref.8) and should normally yield no media fill contamination. For example, a single contaminated unit in a 10,000 unit media fill batch should be fully investigated, but is normally not considered on its own to be sufficient cause for line revalidation. However, intermittent incidents at this media fill contamination level can be indicative of a persistent low level contamination problem. Accordingly, any pattern of media fill batches with such low level contamination should be comprehensively investigated and would be cause for line revalidation.

A firm's use of media fill acceptance criteria allowing infrequent contamination does not mean that a distributed lot of drug product purporting to be sterile may contain a non-sterile unit. The purpose of an aseptic process is to prevent any contamination. A manufacturer is fully liable for the shipment of any non-sterile unit, an act that is prohibited under the FD&C Act. FDA also recognizes that there might be some scientific and technical limitations on how precisely and accurately validation can characterize a system of controls intended to exclude contamination.

As with any validation batch, it is important to note that "invalidation" of a media fill run should be a rare occurrence. A media fill lot should be aborted only under circumstances in which written procedures require commercial lots to be equally handled. Supporting documentation and justification should be provided in such cases.

B. Filtration Efficacy

Filtration is a common method of sterilizing drug product solutions. An appropriate sterilizing grade filter is one which reproducibly removes all microorganisms from the process stream, producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or smaller. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate "worst case" production conditions regarding the size of microorganisms in the material to be filtered and integrity test results of the filters used for the study. The microorganisms should be small enough to both challenge the nominal porosity of the filter and simulate the smallest microorganism that may occur in production. The microorganism *Brevundimonas diminuta* (ATCC 19146) when properly grown, harvested and used, can be satisfactory in this regard because it is one of the smallest bacteria (0.3 micron mean diameter). Bioburden of unsterilized bulk solutions should be determined, in order to trend the characteristics of potentially contaminating organisms. In certain cases, when justified as equivalent or better than use of *Brevundimonas diminuta*, it may be appropriate to conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in the challenge is important because a filter can contain a number of pores larger than the nominal rating which have potential to allow passage of microorganisms (Ref. 9). The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases (Ref. 10). A challenge concentration of at least 10^7 organisms per cm^2 of

effective filtration area of *B. diminuta* is generally used. A commercial lot's actual influent bioburden should not include microorganisms of a size and/or concentration that would present a challenge beyond that considered by the validation study.

Direct inoculation into the drug formulation provides an assessment of the effect of drug product on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta* into products with inherent bactericidal activity or into oil-based formulations can lead to erroneous conclusions. When sufficiently justified, the effects of the product formulation on the membrane's integrity can be assessed using an appropriate alternate method. For example, the drug product could be filtered in a manner in which the worst-case combination of process specifications and conditions are simulated. This step could be followed by filtration of the challenge organism for a significant period of time, under the same conditions, using an appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial component) as the vehicle. Any divergence from a simulation using the actual product and conditions of processing should be justified. Factors which can affect filter performance normally include: (1) viscosity of the material to be filtered; (2) pH; (3) compatibility of the material or formulation components with the filter itself; (4) pressures; (5) flow rates; (6) maximum use time; (7) temperature; (8) osmolality; (9) and the effects of hydraulic shock. When designing the validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted using the worst case conditions, such as maximum filter use time and pressure (Ref. 11). Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is essential that laboratory experiments simulate actual production conditions. The specific type of filter used in commercial production should be evaluated in filter validation studies. When the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user to review the validation data on the efficacy of the filter in producing a sterile effluent. The data should be applicable to the user's products and conditions of use because filter performance may differ significantly for various conditions and products.

After a filtration process is properly validated for a given product, process and filter, it is important to ensure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. Sterilizing filters should be routinely discarded after processing of a single batch. Normally, integrity testing of the filter is performed after the filter unit is assembled and sterilized prior to use. It is important that the integrity testing be conducted after filtration in order to detect any filter leaks or perforations that might have occurred during the filtration. "Forward flow" and "bubble point" tests, when appropriately employed, are two acceptable integrity tests. A production filter's integrity test specification should be consistent with data generated during filtration efficacy studies.

C. Sterilization of Equipment and Container/Closures

In order to maintain sterility, equipment surfaces that contact sterilized drug product or sterilized container/closure surfaces must be sterile so as not to alter purity of the drug (211.63 and 211.113). Those surfaces that are in the vicinity of sterile product or container-closures, but do

not directly contact product should also be rendered sterile where reasonable contamination potential exists. It is as important in aseptic processing to properly validate the processes used to sterilize such critical equipment as it is to validate processes used to sterilize the drug product and its container/closure. Moist heat and dry heat sterilization are most widely used and the primary processes discussed in this document. It should be noted that many of the heat sterilization principles discussed in this document are also applicable to other sterilization methods.

Sterility of aseptic processing equipment (e.g., stopper hoppers) should be maintained by batch-by-batch sterilization. Following sterilization of equipment, containers, or closures, any transportation or assembly needs to be performed in a manner in which its sterile state is protected and sustained, with adherence to strict aseptic methods.

1. Sterilizer Qualification and Validation

Validation studies should be conducted demonstrating the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis. For both the validation studies and routine production, use of a specified load configuration should be documented in the batch records.

Unevacuated air's insulating properties prevent moist heat from penetrating or heating up materials, and achieving the lethality associated with saturated steam. Consequently, there is a far slower thermal energy transfer and rate of kill from the dry heat in insulated locations in the load. It is important to remove all of the air from the autoclave chamber during the sterilization cycle. Special attention should be given to the nature or type of the materials to be sterilized and the placement of biological indicator within the sterilization load. D-value of the biological indicator can vary widely depending on the material (e.g., glass versus Teflon) to be sterilized. Difficult to reach locations within the sterilizer load and specific materials should be an important part of the evaluation of sterilization cycle efficacy. Thereafter, requalification/revalidation should continue to focus on load areas identified as the most difficult to penetrate or heat (e.g., worst-case locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, stopper load).

The formal program providing for regular (i.e., semiannual, annual) revalidation should consider the age of the sterilizer and its past performance. Change control procedures should adequately address issues such as a load configuration change or a modification of the sterilizer.

a) Qualification: Empty Chamber

Temperature distribution studies evaluate numerous locations throughout an empty sterilizing unit (e.g., steam autoclave, dry heat oven) or equipment train (e.g., large tanks, immobile piping). It is important that these studies assess temperature uniformity at various locations throughout the sterilizer to identify potential "cold spots" where there can be insufficient heat to attain sterility. These heat uniformity or "temperature mapping" studies should be conducted by placing calibrated temperature measurement

devices in numerous locations throughout the chamber.

b) Validation: Loaded Chamber

Heat penetration studies should be performed using the established sterilizer load(s). Validation of the sterilization process with a loaded chamber demonstrates the effects of loading on thermal input to the items being sterilized, and may identify "cold spots" where there is insufficient heat to attain sterility. The placement of biological indicators (BI) at numerous positions in the load, including the most difficult to sterilize places, is a direct means of demonstrating the efficacy of any sterilization procedure. In general, the thermocouple (TC) is placed adjacent to the BI so as to assess the correlation between microbial lethality and thermal input. Validation of sterilization can be performed using a partial or half-cycle approach. In some cases, the "bioburden" based cycle is used for sterilization validation. For further information on validation using moist heat sterilization, please refer to FDA guidance, "Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products" (November, 1994).

Sterilization cycle specifications are based upon the delivery of adequate thermal input to the slowest to heat locations. When determining which articles are most difficult to sterilize, special attention should be given to the sterilization of filters. For example, some filter installations in piping cause a significant pressure differential across the filter, resulting in a significant temperature drop on the downstream side. Biological indicators should be placed at appropriate downstream locations of this equipment to determine if the drop in temperature affects the thermal input at these sites. Established load configuration should be part of batch record documentation. A sterility assurance level of 10^{-6} or better should be demonstrated for the sterilization process.

2. Equipment Controls and Instrument Calibration

For both validation and routine process control, the reliability of the data generated by sterilization cycle monitoring devices should be considered to be of the utmost importance. Devices that measure cycle parameters should be routinely calibrated. Written procedures should be established to ensure these devices are maintained in a calibrated state. For example:

- Temperature monitoring devices for heat sterilization should be calibrated at suitable intervals, as well as before and after validation runs.
- Devices used to monitor dwell time in the sterilizer should be periodically calibrated.
- The microbial count and D-value of a biological indicator should be confirmed before a validation study.
- Instruments used to determine the purity of steam should be calibrated.
- For dry heat depyrogenation tunnels, devices (e.g. sensors and transmitters) used to measure belt speed should be routinely calibrated.

Sterilizing equipment should be properly maintained to allow for consistently satisfactory function. Evaluation of sterilizer performance attributes such as equilibrium ("come up") time studies should be helpful in assessing if the unit continues to operate properly.

966
967 # X. LABORATORY CONTROLS
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Section 211.160 (General Requirements) states "Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity."

Sections 211.165 and 211.194 require that validation of test methods be established and documented.

Sections 211.22 (c) states that "the quality control unit shall have the responsibility for approving or rejecting all procedures and specifications impacting on the identity, strength, quality, and purity of the drug product."

Section 211.42 requires, for aseptic processes, the establishment of a "system for monitoring environmental conditions."

Section 211.56 requires, "written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities." The "written procedures shall be designed to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug products and shall be followed." Section 211.113 (b) requires that "appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed."

Section 211.192 states that "all drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved, written procedures before a batch is released or distributed."

969
970 ## A. Environmental Monitoring
971
972 *1. General Written Program*
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974 In aseptic processing, one of the most important laboratory controls is the establishment of an
975 environmental monitoring program. This monitoring provides meaningful information on the
976 quality of the aseptic processing environment when a given batch is being manufactured as well
977 as environmental trends of the manufacturing area. An adequate program identifies potential
978 routes of contamination, allowing for implementation of corrections before product
979 contamination occurs (211.42 and 211.113).
980
981 Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-
982 defined written program and validated methods. The monitoring program should cover all
983 production shifts and include air, floors, walls, and equipment surfaces, including the critical
984 surfaces in contact with product and container/closures. Written procedures should include a list
985 of locations to be sampled. Sample timing, frequency, and location should be carefully selected
986 based upon its relationship to the operation performed. Samples should be taken throughout the
987 aseptic processing facility (e.g., aseptic corridors; gowning rooms) using appropriate,
988 scientifically sound sampling procedures, standards, and test limits.
989

Locations posing the most microbiological risk to the product are a critical part of the program. It is especially important to monitor the microbiological quality of the aseptic processing clean zone to determine whether or not aseptic conditions are maintained during filling/closing activities. Critical surfaces which contact sterile product should be sterile. Critical surface sampling should be performed at the conclusion of the aseptic processing operation to avoid direct contact with sterile surfaces during processing. Air and surface samples should be taken at the actual working site and at locations where significant activity or product exposure occurs during production.

Environmental monitoring methods do not always recover microorganisms present in the sampled area. In particular, low level contamination can be particularly difficult to detect. Because of the likelihood of false negatives, consecutive growth results are only one type of adverse trend. Increased incidence of contamination over a given period in comparison to that normally detected is an equally significant trend to be tracked.

All environmental monitoring locations should be described in SOPs with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address areas such as: (1) frequency of sampling; (2) when the samples are taken (i.e., during or at the conclusion of operations); (3) duration of sampling; (4) sample size (e.g., surface area, air volume); (5) specific sampling equipment and techniques; (6) alert and action limits; and (7) appropriate response to deviations from alert or action limits.

2. Establishing Limits and a Trending Program

Microbiological monitoring limits should be established based on the relationship of the sampled location to the operation. The limits should be based on the need to maintain adequate microbiological control throughout the entire sterile manufacturing facility. One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitization procedure studies in developing monitoring limits.

Microbiological environmental monitoring should include both alert and action limits. Each individual sample result should be evaluated for its significance by comparing to the alert or action limits. Averaging of results can mask unacceptable localized conditions. A result at the alert limit urges attention to the approaching action conditions. A result at the action level should prompt a more thorough investigation. Written procedures should be established, detailing data review frequency, identification of contaminants, and actions to be taken. The quality control unit should provide routine oversight of near term (e.g., daily, weekly, monthly, quarterly) and long term trends in environmental and personnel monitoring data.

Trend reports should include data generated by location, shift, lot, room, operator, or other search parameters. The quality control unit is responsible for producing specialized data reports (e.g., a search on a particular atypical isolate over a year period) in order to investigate results beyond established limits and identify any appropriate follow-up actions. In addition to microbial counts beyond alert and action limits, the presence of any atypical microorganisms in the cleanroom environment should be investigated, with any appropriate corrective action promptly implemented.

Written procedures should define the system whereby the most responsible managers are regularly informed and updated on trends and investigations.

3. Sanitization Efficacy

The suitability, efficacy, and limitations of sanitization agents should be assessed with their implementation for use in clean areas. The effectiveness of these sanitization procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces (i.e., via obtaining samples before and after sanitization).

Upon preparation, disinfectants should be rendered sterile, and used for a limited time, as specified by written procedures. Disinfectants should retain efficacy against the normal microbial flora and be effective against spore-forming microorganisms. Many common sanitizers are ineffective against spores, for example, 70% isopropyl alcohol is not effective against *Bacillus,* spp. spores. A sporicidal agent should be used regularly to prevent contamination of the manufacturing environment with otherwise difficult to eradicate spore forming bacteria or fungi.

After the initial assessment of sanitization procedures, ongoing sanitization efficacy should be frequently monitored through specific provisionsin the environmental monitoring program, with a defined course of action in the event samples are found to exceed limits.

4. Monitoring Methods

Acceptable methods of monitoring the microbiological quality of the environment include:

a. Surface Monitoring-
Environmental monitoring should include testing of various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, ceilings, and equipment should be tested on a regular basis. Routinely used for such tests are touch plates, swabs, and contact plates. Other surfaces in controlled areas should be tested to show the adequacy of cleaning and sanitizing procedures.

b. Active Air Monitoring-
The method of assessing the microbial quality of air should involve the use of "active" devices such as slit to agar samplers, , those using liquid impingement and membrane filtration, or centrifugal samplers. Each device has certain advantages and disadvantages, although all allow a quantitative testing of the number of organisms per volume of air sampled. The use of such devices in aseptic areas is considered an essential part of evaluating the environment during each production shift, at carefully chosen critical locations. Manufacturers should be aware of a device's air monitoring capabilities, and should determine suitability of any new or current devices with respect to sensitivity and limit of quantification.

c. Passive Air Monitoring (Settling Plates)-

Another method is the use of passive air samplers such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). These settling plates lack value as quantitative air monitors because only microorganisms that settle onto the agar surface will be detected. Their value as qualitative indicators in critical areas is enhanced by positioning plates in locations posing the greatest risk of product contamination. As part of methods validation, the quality control laboratory should evaluate what media exposure conditions optimize recovery of low levels of environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or high airflows), which inhibits recovery of microorganisms. The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples.

B. Microbiological Media and Identification

The environmental monitoring program should include routine characterization of recovered microorganisms. Monitoring of critical and immediately surrounding areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level.

In some cases, environmental trending data has revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser-controlled areas. To detect such trends, an adequate program of differentiating microorganisms in lesser-controlled environments (e.g., Class 100,000) should be in place. At minimum, the program should require species (or, where appropriate, genus) identification of microorganisms in ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective). Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for the associated investigation.

The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of monitoring the state of environmental control. Consistent methods will yield a database that allows for sound data comparisons and interpretations. The microbiological culture media used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and molds) as well as bacteria, and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours. Total combined yeast and mold count is generally obtained by incubating at 20 to 25°C for 5 to 7 days.

Incoming lots of environmental monitoring media should include positive and negative controls. Growth promotion testing should be performed on all lots of prepared media. Where appropriate, inactivating agents should be used to prevent inhibition of growth by clean room disinfectants.

C. Pre-filtration Bioburden

For any parenteral manufacturing process, pre-filtration bioburden should be minimal. In addition to increasing the challenge to the sterilizing filter, high bioburden can contribute endotoxin or other impurities to the drug formulation. An in-process limit for bioburden level for each formulated product (generally sampled immediately preceding sterile filtration) should be established.

D. Particulate Monitoring

Routine particle monitoring is useful in detecting significant deviations in air cleanliness from qualified processing norms (e.g., clean area classification). A result outside the established specifications at a given location should be investigated consistent with the severity of the "excursion." Appropriate corrective action should be implemented to prevent future deviations.

See Section IV.A for additional guidance on particulate monitoring.

XI. STERILITY TESTING

Section 211.167 (Special Testing Requirements) states: "For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

Section 211.165 states "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product ...prior to release."

Section 211.165(e) requires methods for testing to be validated as reliable and reproducible (e.g., bacteriostasis/fungistasis, method robustness, etc.), stating: "The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with Sec. 211.194(a)(2)."

Section 211.110 requires, in part, that sampling procedures are established in order to ensure batch uniformity: The "control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product."

Section 211.160 requires the establishment of sound and appropriate sampling plans which are representative of the batch.

Section 210 defines "representative sample" as one based on rational criteria that provide an "accurate portrayal" of the material or batch being sampled.

Section 211.180 states a review of, "at least annually, the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures." Investigations conducted under Section 211.192 for each drug product are required to be addressed within this annual review.

Certain aspects of sterility testing are of particular importance, including control of the testing environment, understanding the test limitations, and the investigation of manufacturing systems following a positive test.

The testing laboratory environment should employ facilities and controls comparable to those used for filling/closing operations. Poor or deficient sterility test facilities or controls can result in a high rate of test failures. If production facilities and controls are significantly better than those for sterility testing there is the danger of attributing the cause of a positive sterility test result to the faulty laboratory even when the product tested could have, in fact, been non-sterile. Therefore, some manufacturing deficiency may go undetected. The use of isolators to perform sterility testing is a well-established means for minimizing false positives.

A. Choice of Methods

Sterility testing methodologies are required to be accurate and reproducible, in accord with 211.194 and 211.165. The methodology selected should present the lowest potential for yielding a false positive. The USP specifies membrane filtration as the method of choice, when feasible.

As a part of methods validation, appropriate bacteriostasis/fungistasis testing should be conducted. Such testing should demonstrate reproducibility of the method in recovering each of a panel of representative microorganisms. Study documentation should include evaluation of whether microbial recovery from inoculated controls and product samples is comparable throughout the incubation period. If growth is inhibited, modifications (e.g., increased dilution, additional membrane filter washes, addition of inactivating agents) in the methodology should be implemented to optimize recovery. Ultimately, methods validation studies should demonstrate that the methodology does not provide an opportunity for "false negatives."

B. Media

It is essential that the media used to perform sterility testing be rendered sterile and demonstrated as growth promoting.

C. Personnel

Personnel performing sterility testing should be qualified and trained for the task. A written program should be in place to regularly update training of personnel and confirm acceptable sterility testing practices.

D. Sampling and Incubation

Sterility tests are limited in their ability to detect low levels of contamination. For example, statistical evaluations indicate that the USP sterility test sampling plan has been described by USP as "only enabling the detection of contamination in a lot in which 10% of the units are contaminated about nine times out of ten in making the test" (Ref. 12). To further illustrate, if a

10,000 unit lot with a 0.1% contamination level was sterility tested using 20 units, there is a 98% chance that the batch would pass the test.

This limited sensitivity makes it necessary to ensure that for batch release purposes an appropriate number of units are tested and that the samples uniformly represent the:

(1) Entire batch-
Samples should be taken at the beginning, middle, and end of the aseptic processing operation;
(2) Batch processing circumstances-
Samples should be taken in conjunction with processing interventions or excursions.

Because of the limited sensitivity of the test, any positive result is considered a serious CGMP issue and should be thoroughly investigated.

E. Investigation of Sterility Positives

Care should be taken in the performance of the sterility test to preclude any activity that allows for possible sample contamination. When microbial growth is observed, the lot should be considered to be non-sterile. It is inappropriate to attribute a positive result to laboratory error on the basis of a retest that exhibits no growth.[8] The evaluation of a positive sterility test result should include an investigation to determine whether the growth observed in the test arose from product contamination or from laboratory error.

Although it is recognized that such a determination may not be reached with absolute certainty, it is usually possible to acquire persuasive evidence showing that causative laboratory error is absent. When available evidence is inconclusive, batches should be rejected as not conforming to sterility requirements.

It would be difficult to support invalidation of a positive sterility test. Only if conclusive and documented evidence clearly shows that the contamination occurred as part of testing should a new test be performed.

After considering all relevant factors concerning the manufacture of the product and testing of the samples, the comprehensive written investigation should include specific conclusions, and identify corrective actions. The investigation's persuasive evidence of the origin of the contamination should be based upon at least the following:

1. Identification (speciation) of the organism in the sterility test. Identification of the sterility test isolate(s) should be to the species level. Microbiological monitoring data should be reviewed to determine if the organism is also found in laboratory and production environments, personnel, or product bioburden.

[8] Underscoring this regulatory standard, USP XXV, section <71>, states that an initial positive test is invalid only in an instance in which "microbial growth can be without a doubt ascribed to" laboratory error (as described in the monograph).

2. Record of laboratory tests and deviations. Review of trends in laboratory findings can help to eliminate or implicate the laboratory as the source of contamination. If the organism is seldom found in the laboratory environment, then product contamination is likely. If the organism is found in laboratory and production environments, it can indicate product contamination.

   The proper handling of deviations is an essential aspect of laboratory control. When a deviation occurs during sterility testing, it should be documented, investigated, and remedied. If any deviation is considered to have compromised the integrity of the sterility test, the test should be invalidated immediately without incubation.

   Deviation and sterility test positive trends should be evaluated periodically (e.g., quarterly, annually) to provide an overview of operations. A sterility positive result can be viewed as indicative of production or laboratory problems and should be investigated globally since such problems often can extend beyond a single batch.

   In order to more accurately monitor potential contamination sources, it is useful to keep separate trends by product, container type, filling line, and personnel. Where the degree of sterility test sample manipulation is similar for a terminally sterilized product and an aseptically processed product, a higher rate of initial sterility failures for the latter should be taken as indicative of aseptic processing production problems. See Section IX.A, Process Simulations, which includes similar issues that are investigated as part of a media fill failure investigation.

   Microbial monitoring of the laboratory environment and personnel over time can also reveal trends that are informative. Upward trends in the microbial load in the laboratory should be promptly investigated as to cause, and corrected. In some instances, such trends can appear to be more indicative of laboratory error as a possible source of a sterility test failure.

   Where a laboratory has a good track record with respect to errors, this history can help remove the lab as a source of contamination since chances are higher that the contamination arose from production. However, the converse is not true. Specifically, where the laboratory has a poor track record, firms should not assume that the contamination is automatically more attributable to the error in laboratory and consequently overlook a genuine production problem. Accordingly, all sterility positives should be thoroughly investigated.

3. Monitoring of production area environment. Of particular importance is trend analysis of microorganisms in the critical and immediately adjacent area. Trends are an important tool in investigating the product as the possible source of a sterility failure. Consideration of environmental microbial loads should not be limited to results of monitoring the production environment for the lot, day, or shift associated with the suspect lot. For example, results showing little or no recovery of microorganisms can be misleading, especially when preceded or followed by a finding of an adverse trend or atypically high microbial counts. It is therefore important to look at both short and long term trend analysis.

4. Monitoring of Personnel. Daily personnel monitoring data and associated trends should be reviewed and can in some cases strongly indicate a route of contamination. The adequacy of personnel practices and training should also be considered.

5. Product pre-sterilization bioburden. Trends in product bioburden should be reviewed (counts and identity). Adverse bioburden trends occurring during the time period of the test failure should be considered in the investigation.

6. Production record review. Complete batch and production control records should be reviewed to detect any signs of failures or anomalies which could have a bearing on product sterility. For example, the investigation should evaluate batch and trending data that indicate whether utility/support systems (e.g., HVAC, WFI) are functioning properly. Records of air quality monitoring for filling lines should show a time at which there was improper air balance, an unusual high particulate count, etc.

7. Manufacturing history. The manufacturing history of the product or similar products should be reviewed as part of the investigation. Past deviations, problems, or changes (e.g., process, components, equipment) are among the factors that can provide an indication of the origin of the problem.

XII. BATCH RECORD REVIEW: PROCESS CONTROL DOCUMENTATION

Sections 211.100, 211.186, and 211.188 address documentation of production and control of a batch, including recording various production and process control activities at the time of performance. Section 211.100 (b) requires a documented record and evaluation of any deviation from written procedures.

Section 211.192 states that "All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup."

Maintaining process and environmental control is a daily necessity for an aseptic processing operation. The requirement for review of all batch records and data for conformance with written procedures, operating parameters, and product specifications prior to arriving at the final release decision for an aseptically processed batch calls for an overall review of process and system performance for that given cycle of manufacture. All in-process data must be included with the batch record documentation per Section 211.188. Review of environmental monitoring data as well as other data relating to the acceptability of output from support systems (e.g., HEPA / HVAC, WFI, steam generator) and proper functioning of equipment (e.g., batch alarms report; integrity of various filters), should be viewed as essential elements of the batch release decision.

While interventions and/or stoppages are normally recorded in the batch record, the manner of documenting these occurrences varies. In particular, line stoppages and any unplanned interventions should be sufficiently documented in batch records with the associated time and duration of the event. In general, there is a correlation between product (or container-closure) dwell time in the aseptic processing zone and the probability of contamination. Sterility failures can be attributed to atypical or extensive interventions that have occurred as a response to an undesirable event during the aseptic process. Written procedures describing the need for line clearances in the event of certain interventions, such as machine adjustments and any repairs, should be established. Such interventions should be documented with more detail than minor events. Interventions that result in substantial activity near exposed product/container-closures or that last beyond a reasonable exposure time should, where appropriate, result in a local or full line clearance

Any disruption in power supply, however momentary, during aseptic processing is a manufacturing deviation and must be included in batch records (211.100, 211.192).

APPENDIX 1: ASEPTIC PROCESSING ISOLATORS

An emerging aseptic processing technology uses isolation systems to minimize the extent of personnel involvement and to separate the external cleanroom environment from the aseptic processing line. A well designed positive pressure barrier isolator, supported by adequate procedures for its maintenance, monitoring, and control, appears to offer an advantage over classical aseptic processing, including fewer opportunities for microbial contamination during processing. However, users should not adopt a "false sense of security" with these systems. Manufacturers should be also aware of the need to establish new procedures addressing issues unique to these systems.

A) Maintenance

1. General

Isolator systems have a number of special maintenance requirements. While no isolator unit forms an absolute seal, very high integrity can be achieved in a well-designed unit. However, a leak in any of certain components of the system can constitute a significant breach of integrity. The integrity of gloves, half-suits, seams, gaskets, and seals require daily attention and a comprehensive preventative maintenance program. Replacement frequencies should be established in written procedures that require changing parts before they breakdown or degrade.

2. Glove Integrity

A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and a critical breach of isolator integrity. The choice of durable glove materials, coupled with a well-justified replacement frequency, are two aspects of good manufacturing practice that should be addressed. With every use, gloves should be visually evaluated for any macroscopic physical defect. Mechanical integrity tests should also be performed routinely. This attentive preventative maintenance program is necessary to prevent use of gloves lacking integrity that would place the sterile product at risk. When such a breach is discovered, the operation should be terminated.

Due to the potential for microbial migration through microscopic holes in gloves and the lack of a highly sensitive glove integrity test, the inner part of the installed glove should be sanitized regularly and the operator should also wear a second pair of thin gloves.

B) Design

1. Airflow

The design of an aseptic processing isolator normally employs unidirectional airflow that sweeps over and away from exposed sterile materials, avoiding any turbulence or stagnant airflow in the area of exposed sterilized materials, product, and container-closures. In most sound designs, air showers over the critical zone once, and then is systematically exhausted. Air handling systems should employ HEPA and/or ULPA filters in series.

2. Materials of Construction

As in any aseptic processing design, suitable materials should be chosen based on durability, as well as ease of cleaning and sterilization. For example, rigid wall construction incorporating stainless steel and glass materials is widely used.

3. Pressure Differential

Isolators that include an open exit portal represent a potential compromise in achieving complete physical separation from the external environment. A positive air pressure differential adequate to achieve this full separation should be employed and supported by qualification studies. Positive air pressure differentials from the isolator to the surrounding environment have largely ranged from approximately 0.07" to 0.2" water gauge. The appropriate minimum pressure differential specification established by a firm will be dependent on the system's design and, when applicable, its exit port. Air balance between the isolator and other direct interfaces (e.g., dry heat tunnel) should also be qualified.

The positive pressure differential should be coupled with appropriate protection at the product egress point(s) in order to overcome the potential for ingress of any airborne particles from the external environment by induction. Induction can result from local turbulent flow causing air swirls or pressure waves that can push extraneous particles into the isolator. Local Class 100 protection at an opening can provide a further barrier to induction of outside air into the isolator.

4. Clean Area Classifications

The interior of the isolator should, at minimum, meet Class 100 standards. The classification of the environment surrounding the isolator should be based on the design of the product interfaces, such as transfer ports and discharge points, as well as the number of transfers into and out of the isolator. A Class 10,000 or Class 100,000 background is appropriate depending on isolator design and manufacturing situations. The area surrounding the isolator should be justified. An isolator should not be located in an unclassified room.

C) Transfer of Materials/Supplies

The ability to maintain integrity and sterility of an isolator is impacted by the design of transfer ports. Various adaptations, of differing capabilities, allow for the transfer of supplies into and out of the isolator.

1. Introduction:

Multiple material transfers are generally made during the processing of a batch. Frequently, transfers are performed via direct interface with a decontaminating transfer isolator or dry heat depyrogenation tunnel with balanced airflow. Such provisions, if well designed, help ensure that microbiological ingress does not result from the introduction of supplies. Properly operated RTPs (rapid transfer ports) are also generally considered to be an effective transfer mechanism. The number of transfers should be kept to a minimum because the risk of ingress of contaminants increases with each successive material transfer.

Some transfer ports can have significant limitations, including marginal decontaminating capability (e.g., ultraviolet) or a design that would compromise isolation by allowing ingress of air from the surrounding room. In the latter case, localized HEPA-filtered laminar airflow cover in the area of such a port should be implemented.

2. Discharge:

Isolators often include a "mousehole" or other exit port through which product is discharged, opening the isolator to the outside environment. The mousehole represents a potential route of contamination. Sufficient overpressure should be supplied and monitored on a continuous basis at this location to ensure that isolation is maintained.

D) Decontamination

1. Surface Exposure

Written procedures for decontamination of the isolator should be established. The decontamination process should provide full exposure of all isolator surfaces to the chemical agent. For example, in order to facilitate contact with the sterilant, the glove apparatus should be fully extended with glove fingers separated during the decontamination cycle.

2. Efficacy

A decontamination method should be developed which renders the inner surfaces of the isolator free of viable microorganisms. Decontamination can be accomplished using a number of vaporized agents, although these agents possess limited capability to penetrate obstructed or covered surfaces. Process development and validation studies should include a thorough determination of cycle capability. The characteristics of these agents generally preclude the reliable use of statistical methods (e.g., fraction negative) to determine process lethality. An appropriate, quantified BI challenge should be placed on various materials and in many locations throughout the isolator, including difficult to reach areas. Cycles should be developed with an appropriate margin of extra kill to provide confidence in robustness of the decontamination processes. For most production applications, demonstration of a six-log reduction of the challenge BI is recommended.

The uniform distribution of the defined concentration of decontaminating agent should also be evaluated concurrent with these studies. Chemical indicators may also be useful as a qualitative tool to show that the decontaminating agent reached a given location.

3. Frequency

While isolators vary widely in design, their interior and content should be designed to be frequently decontaminated. If an isolator is to be used for multiple days between decontamination cycles, the frequency adopted should include a built-in safety margin

and be well justified. This frequency, established during validation studies, should be reevaluated and increased if production data indicate any deterioration of the microbiological quality of the isolator environment.

A breach of isolator integrity (e.g., power failure, glove/seam tear, other air leaks, valve failure, out of specification pressure) should lead to a decontamination cycle. Breaches of integrity should be investigated and any product that may have been impacted by the breach rejected.

E) Filling Line Sterilization

In order to ensure sterility of product contact surfaces from the start of each operation, the entire path of the sterile liquid stream should be sterilized. In addition, loose materials or equipment to be used within the isolator should be chosen based on their ability to withstand steam sterilization (or equivalent method). It is expected that any materials that can be subjected to a steam sterilization cycle will, in fact, be autoclaved.

F) Environmental Monitoring

An appropriate environmental monitoring program should be established which routinely ensures acceptable microbiological quality of air, surfaces, and gloves (or half-suits) as well as particulate levels, within the isolator. Air quality should be monitored periodically during each shift. As an example, the exit port should be monitored for particulates to detect any unusual results.

G) Personnel

While cleanroom apparel requirements are generally reduced, the contribution of human factor to contamination should not be overlooked. Isolation processes generally include periodic or even frequent use of one or more gloves for aseptic manipulations and handling of component transfers into and out of the isolator. Contaminated gloves can lead to product non-sterility. This concern is heightened because locations on gloves, sleeves, or half suits can be among the more difficult to reach places during surface sterilization. Meticulous aseptic technique standards must be observed (211.113).

APPENDIX 2: BLOW-FILL- SEAL TECHNOLOGY

Blow-fill-seal (BFS) technology is an automated process by which containers are formed, filled, and sealed in a continuous operation. This manufacturing technology includes economies in container-closure processing and reduced human intervention, and is often used for filling and packaging of ophthalmics and, less frequently, for injectables. This section discusses some of the critical control points of this technology. Except where otherwise noted below, the aseptic processing standards discussed elsewhere in this document should be applied to Blow Fill Seal technology.

A) Equipment Design and Air Quality

A BFS machine operates by 1) heating a plastic polymer resin; 2) extruding it to form a parison (a tubular form of the hot resin); 3) cutting the parison with a high temperature knife; 4) moving the parison under the blow-fill needle (mandrel); 5) inflating it to the shape of the mold walls; 5) filling the formed container with the liquid product; 6) removing the mandrel; 7) sealing. Throughout this operation sterile-air is used, for example, to form the parison and inflate it prior to filling. In most operations, the three steps which pose greatest potential for exposure to particle contamination and/or surrounding air are those in which: the parison is cut; the parison is moved under the blow-fill mandrel; and the mandrel is removed (just prior to sealing).

BFS machinery and its surrounding barriers should be designed to prevent potential for extraneous contamination. As with any aseptic processing operation, it is critical that contact surfaces be sterile. A validated steam-in-place cycle should be used to sterilize the equipment path through which the product is conveyed. In addition, any other surface (e.g., above or nearby) that has potential to contaminate the sterile product needs to be sterile.

The classified environment surrounding BFS machinery should generally meet Class 10,000 standards, but special design provisions (e.g., isolation technology) can justify an alternate classification. HEPA-filtered or sterile air provided by membrane filters is necessary in the critical zone in which sterile product or materials are exposed (e.g., parison formation, container molding/filling steps). Air in the critical zone should meet Class 100 microbiological standards. A well-designed BFS system should also normally achieve Class 100 particulate levels.

Equipment design should incorporate specialized measures to reduce particulate levels. In contrast to non-pharmaceutical applications using BFS machinery, control of air quality (i.e., particulates) is critical for sterile drug product manufacture. Particles generated during the plastic extrusion, cutting, and sealing processes provide a potential means of transport for microorganisms into open containers prior to sealing. Provisions for carefully controlled airflow could protect the product by forcing generated particles outward while preventing any ingress from the adjacent environment. Furthermore, designs separating the filling zone from the surrounding environment are important in ensuring product protection. Barriers, pressure vacuums, microenvironments, and appropriately directed high velocities of sterile air have been found useful in preventing contamination (Ref. 13). Smoke studies and multi-location particulate data are vital when performing qualification studies to assess whether proper particulate control dynamics have been achieved throughout the critical area.

In addition to suitable design, an adequate preventative maintenance program should be established. For example, because of its potential to contaminate the sterile drug product, the integrity of the boiling system (e.g., mold plates, gaskets) should be carefully monitored and maintained.

B) Validation/Qualification

Advantages of BFS processing are known to include rapid container/closure processing and minimized interventions. However, a properly functioning process is necessary to realize these

advantages. Equipment qualification/requalification and personnel practices should be given special attention. Equipment sterilization, media fills, polymer sterilization, endotoxin removal, product-plastic compatibility, forming/sealing integrity, and unit weight variation are among the key issues that should be covered by validation/qualification studies.

Appropriate data should ensure that BFS containers are sterile and non-pyrogenic. This can generally be achieved by validating that time-temperature conditions of the extrusion process destroy the worst-case endotoxin load on the polymeric material.

The plastic polymer material chosen should be pharmaceutical grade, safe, pure, and pass USP criteria for plastics. Polymer suppliers should be qualified and monitored for raw material quality.

C) Batch Monitoring and Control

In-process monitoring should include various control parameters (e.g., container weight variation, fill weight, leakers, air pressure, etc.) to ensure ongoing process control. Environmental monitoring is particularly important. Samples should be taken during each shift at specified locations under dynamic conditions. Due to the generation of high levels of particles near the exposed drug product, continuous monitoring of particles can provide valuable data relative to the control of a blow-fill-seal operation.

Container-closure defects can be a major problem in control of a BFS operation. It is necessary for the operation to be designed and set-up to uniformly manufacture leak-proof units. As a final measure, inspection of each unit of a batch should employ a reliable, sensitive final product examination capable of detecting a defective unit (e.g., "leakers"). Significant defects due to heat or mechanical problems, such as mold thickness, container/closure interface deficiencies, poorly formed closure, or other deviations should be investigated in accord with Sections 211.100 and 211.192.

APPENDIX 3: PROCESSING PRIOR TO FILLING/SEALING OPERATIONS

The purpose of this appendix is to supplement the guidance provided in this document with information on products regulated by CBER or CDER that are subject to aseptic processing from early in the manufacturing process, or that require aseptic processing through the entire manufacturing process, due to their inability to be sterilized. The scope of this appendix includes aseptic processing activities that take place prior to the filling and sealing of the finished drug product. Special considerations include those for:

A) Aseptic processing from early manufacturing steps

Due to their nature, some products undergo aseptic processing at some or all manufacturing steps preceding the final product closing step. There is a point in the process after which a product can no longer be rendered sterile by filtration, and the product is handled aseptically in all subsequent steps. Some products are formulated aseptically because the formulated product cannot be

sterilized by filtration. For example, products containing aluminum adjuvant are formulated aseptically because once they are alum adsorbed, they cannot be sterile filtered.

When a product is processed aseptically from early steps, the product and all components or other additions are rendered sterile prior to entering the manufacturing process. It is critical that all transfers, transports, and storage stages are carefully controlled at each step of the process to maintain sterility of the product.

Procedures that expose the product or product contact equipment surfaces to the environment, such as aseptic connections, should be performed under unidirectional airflow in a Class 100 environment. The environment of the room surrounding the Class 100 environment should be class 10,000 or better. Microbiological and particulate monitoring should be performed during operations. Microbial surface monitoring should be performed at the end of operations, but prior to cleaning. Personnel monitoring should be performed in association with operations.

Process simulation studies should be designed to incorporate all conditions, product manipulations, and interventions that could impact on the sterility of the product during manufacturing. The process simulation, from early process steps, should demonstrate that controls over the process are adequate to protect the product during manufacturing. These studies should incorporate all product manipulations, additions, and procedures involving exposure of product contact surfaces to the environment. The studies should include worst-case conditions such as maximum duration of open operations and maximum number of participating operators. However, process simulations do not need to mimic total manufacturing time if the manipulations that occur during manufacturing are adequately represented.

It is also important that process simulations incorporate storage of product or transport to other manufacturing areas. For instance, there should be assurance of bulk vessel integrity for specified holding times. The transport of bulk tanks or other containers should be simulated as part of the media fill. Please refer to Section IX.A for more guidance on media simulation studies. Process simulation studies for the formulation stage should be performed at least twice per year.

B) Aseptic processing of cell-based therapy products (or of products intended for use as cell based therapies)

Cell-based therapy products represent a subset of the products for which aseptic manipulations are used throughout the process. Where possible, closed systems should be used during production. Cell-based therapy products often have short processing times at each manufacturing stage, even for the final product. Often, it is appropriate for these products to be administered to patients before final product sterility testing results are available. In situations where results of final sterility testing are not available before the product is administered, additional controls and testing should be considered. For example, additional sterility tests can be performed at intermediate stages of manufacture, especially after the last manipulation of the product prior to administration. Other tests that may indicate microbial contamination, such as microscopic examination, gram stains, and endotoxin testing should be performed prior to product release.

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13. Price, Jeff, "Blow-Fill-Seal Technology: Part I, A Design for Particulate Control," Pharmaceutical Technology, February, 1998.
14. United States Pharmacopoeia

RELEVANT GUIDANCE DOCUMENTS

Some relevant FDA guidances include:

- Guidance for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Product, 1994
- Guideline for Validation of Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices, 1987
- Guide to Inspections of Lyophilization of Parenterals, 1993
- Guide to Inspections of High Purity Water Systems, 1993
- Guide To Inspections of Microbiological Pharmaceutical Quality Control Laboratories, 1993
- Guide To Inspections of Sterile Drug Substance Manufacturers, 1994
- Pyrogens: Still a Danger; 1979 (Inspection Technical Guide)
- Bacterial Endotoxins/Pyrogens; 1985 (Inspection Technical Guide)
- Heat Exchangers to Avoid Contamination; 1979 (Inspection Technical Guide)

For more information on FDA guidance, see our website at www.fda.gov.

DRAFT GLOSSARY

Air lock- A small room with interlocked doors, constructed to maintain air pressure control between adjoining rooms (generally with different air cleanliness standards). The intent of an aseptic processing airlock is to preclude ingress of particulate matter and microorganism contamination from a lesser controlled area.

Alert Limit- An established microbial or particulate level giving early warning of potential drift from normal operating conditions and which trigger appropriate scrutiny and follow-up to address the potential problem. Alert Limits are always lower than Action Limits.

Action Limit- An established microbial or particulate level which when exceeded should trigger appropriate investigation and corrective action based on the investigation.

Aseptic Processing Facility- Building containing cleanrooms in which air supply, materials, and equipment are regulated to control microbial and particulate contamination.

Aseptic Processing Room- A room in which one or more aseptic activities or processes is performed.

Asepsis- State of control attained by using an aseptic work area and performing activities in a manner that precludes microbiological contamination of the exposed sterile product.

Bioburden- Total number of microorganisms associated with a specific item prior to sterilization.

Barrier- Physical partition that affords aseptic manufacturing zone protection by partially separating it from the surrounding area.

Biological Indicator (BI)- A population of microorganisms inoculated onto a suitable medium (e.g., solution, container/closure) and placed within appropriate sterilizer load locations to determine the sterilization cycle efficacy of a physical or chemical process. The challenge microorganism is selected based upon its resistance to the given process. Incoming lot D-value and microbiological count define the quality of the BI.

Clean Area- An area with defined particulate and microbiological cleanliness standards (e.g., Class 100, Class 10,000 or Class 100,000).

Cleanroom- A room designed, maintained, and controlled to prevent particulate and microbiological contamination of drug products. Such a room is assigned and reproducibly meets an appropriate air cleanliness classification.

Clean Zone- See Clean Area.

Component- Any ingredient intended for use in the manufacture of a drug product, including those that may not appear in the final drug product.

Colony Forming Unit (CFU)- A microbiological term which describes the formation of a single macroscopic colony after the introduction of one (or more) microorganism(s) to microbiological growth media. One colony forming unit is expressed as 1 CFU.

Critical areas - Areas designed to maintain sterility of sterile materials. Sterilized product, container/closures, and equipment may be exposed in critical areas.

Critical surfaces - Surfaces which may come into contact with or directly impact on sterilized product or containers/closures. Critical surfaces are rendered sterile prior to the start of the manufacturing operation and sterility is maintained throughout processing.

Decontamination- A process which eliminates viable bioburden via use of sporicidal chemical agents.

Depyrogenation- A process used to destroy or remove pyrogens (e.g., endotoxin).

D value - The time (in minutes) of exposure to a given temperature that causes a one-log or 90% reduction in the population of a specific microorganism.

Dynamic- Conditions relating to clean area classification under conditions of normal production.

Endotoxin- A pyrogenic product (e.g., lipopolysaccharide) present in the bacterial cell wall. Endotoxin can lead to reactions in patients receiving injections ranging from fever to death.

Gowning Qualification- Program which establishes, both initially and on a periodic basis, the capability of an individual to don the complete sterile gown in an aseptic manner.

HEPA filter- High Efficiency Particulate Air filter with minimum 0.3 micron particle retaining efficiency of 99.97%.

HVAC- Heating, Ventilation, and Air Conditioning.

Intervention- An aseptic manipulation or activity that occurs at the critical zone.

Isolator - A decontaminated unit, supplied with HEPA or ULPA filtered air, which provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding clean room air and personnel).

Laminarity- Unidirectional air flow at a velocity sufficient to uniformly sweep particulate matter away from a critical processing or testing area.

Operator- Any individual participating in the aseptic processing operation, including line set-up, filler, maintenance, or other personnel associated with aseptic line activities.

Overkill sterilization process - A process that is sufficient to provide at least a 12 log reduction of microorganisms having a minimum D value of 1 minute.

Pyrogen- Substance which induces a febrile reaction in a patient.

Sterilizing grade filter- A filter which, when appropriately validated, will remove all microorganisms from a fluid stream, producing a sterile effluent.

Terminal sterilization- The application of a lethal agent to sealed, finished drug products for the purpose of achieving a predetermined sterility assurance level (SAL) of usually less than 10^{-6} (i.e., a probability of a non-sterile unit of greater than one in a million).

ULPA filter- Ultra-Low Penetration Air filter with minimum 0.3 micron particle retaining efficiency of 99.999 %.

Validation- Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

Worst case- A set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, which pose the greatest chance of process or product failure (when compared to ideal conditions). Such conditions do not necessarily induce product or process failure.